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1 **Gain in biodiversity but not in phytostabilization after 3 years of ecological restoration**
2 **of contaminated Mediterranean soils**

3
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15 **Highlights**

16 - Metal and metalloid soil contamination slows down the ecological restoration dynamics

17 - Native plants transplanted in Mediterranean contaminated soils persist after 3 years

18 - Active ecological restoration allows the creation of favorable micro-niches for plants

19 - Metal and metalloid phytostabilization efficiency is not evidenced after 3 years

20

21 **Abstract**

22 Recent studies consider the native flora as a potential source of plant candidates for
23 phytostabilization of metal and metalloid (MM) contaminated soils, but ecological restoration
24 is not the main objective of these researches. However, in contaminated areas,
25 phytostabilization should be considered as a useful tool for ecological restoration. The present
26 study takes stock of 3 years of a Mediterranean pilot site implementation using native plant
27 species to recover plant and microbial communities (diversity and functions) together with
28 soil remediation in the Calanques National Park. To determine the success of this operation,
29 three *in situ* treatments were compared: ecological restoration plots characterized by the
30 handling of physical environment (creation of cultivation terraces) and plants, negative
31 control plots without vegetation but with the same physical environment handling as
32 ecological restoration plots, and positive control plots with natural vegetation and no

33 handling. The results suggest that an ecological restoration trajectory is initiated in the
34 ecological restoration plots, characterized by a partial permanent plant cover. However, there
35 is no evidence of a significant improvement of soil quality (evaluated by soil texture, pH,
36 nutrients and organic carbon contents, cation exchange capacity, microbial biomass and
37 activities) and phytostabilization efficiency after 3 years. Native plant communities and their
38 associated microorganisms may need more time before improving soil quality and MM
39 stabilization under the drastic Mediterranean conditions. Any amendment addition to
40 accelerate restoration and MM immobilization was forbidden in this protected area. Under
41 such conditions, an active restoration need to be carried out in this contaminated area even if
42 resilient dynamics of the native plant communities may sporadically occur over a long period
43 of time.

44

45 **Keywords:** active restoration, field experiment, Mediterranean protected area, metals and
46 metalloids, native plant communities, resilient dynamics

47

48 **1. Introduction**

49 Human activities generate a significant degradation of ecosystems and specifically of soils.
50 Indeed, in 2015, 33 % of soils were considered as moderately or severely degraded by
51 erosion, nutrient depletion, acidification, salinization, compaction and chemical pollution
52 (FAO, 2015). The European Strategy for Biodiversity expects at least 15% of degraded
53 ecosystems to be restored by 2020 (EU, 2011). In this context, some scientists are currently
54 working on ecological restoration of polluted environments (Wong, 2003; Li, 2006)
55 particularly in the Mediterranean region (Heckenroth et al., 2016b). Ecological restoration is
56 the intentional activity that assists ecosystem restoration in order to regain its structural and
57 functional integrity, allowing it to be resistant and/or resilient to normal stress and
58 environmental disturbance levels (SER, 2004). Ecological restoration can be active or passive
59 depending on the level of ecosystem degradation (SER, 2016). Facing weak degradation,
60 stopping the disturbance is often enough for spontaneous plant recolonization of the site (i.e.
61 spontaneous regeneration through passive ecological restoration). When degradation is
62 significant, active restoration operations are required by restoring abiotic components (i.e.
63 assisted regeneration) or both abiotic and biotic components (i.e. reconstruction). In the case
64 of contaminated soils, ecological restoration can be combined with phytoremediation
65 techniques to decontaminate the environment or to avoid pollutants dispersion (Wong, 2003;
66 Li, 2006). Phytostabilization consists of using plants and their associated microorganisms to

67 stabilize contaminants in the roots and/or rhizosphere to limit their transfer *via* erosion,
68 leaching or runoff processes and their bioavailability (Pilon-Smits, 2005) and to promote
69 restoration of contaminated ecosystem especially by metals and metalloids (MM) (Ma et al.,
70 2011; Marchiol et al., 2013). The success of an ecological restoration combined with
71 phytostabilization is based on several criteria. Plant species selection from the local floristic
72 background is essential. The selection must consider plant adaptations to local conditions and
73 their MM tolerance which promote plant survival and development (Baker et al., 2010; Bolan
74 et al., 2011; Cortina et al., 2011). Moreover, permanent and large root systems and plant
75 cover, as well as plant MM immobilization capacities, permit to reduce soil erosion and MM
76 bioavailability, thus limiting contaminant dispersion and transfer into the environment (Pilon-
77 Smits, 2005; Bochet et al., 2006; Bolan et al., 2011). In addition, consideration of biotic
78 interactions is essential, in particular in harsh environments (Padilla and Pugnaire, 2006;
79 Cortina, 2011). Facilitation by nurse plants, favoring micro-niche formation by improving
80 microclimatic and edaphic conditions (Callaway, 2007), permits establishment of other plant
81 species on unfavorable environments (Frérot et al., 2006). Plant associated microorganisms,
82 besides potentially participate in MM immobilization, improve plant MM tolerance by
83 enhancing nutrient and water absorption (Khan, 2005; Ma et al., 2011). Better consideration
84 of soil quality, its contamination level and the contamination spatial heterogeneity are
85 necessary to think how to improve soil properties involved in contaminant retention in order
86 to reduce their bioavailability (Adriano et al., 2004; Bolan et al., 2011; Kumar Yadav et al.,
87 2018). However, the use of ecological restoration tools combined with those of
88 phytostabilization is still very recent and these success criteria are still to be assessed.
89 Moreover, ecological restoration in Mediterranean dry habitats is a challenging topic and
90 implies inventiveness in the developed processes to enhance vegetation establishment
91 (Jaunatre et al., 2012; Rey and Burylo, 2014; Ballesteros et al., 2017). However, in addition to
92 biogeoclimatic constraints framing the tools used, accessibility, pollution, topographical and
93 regulatory constraints may restrict possible actions.

94
95 Mining areas are relevant study cases of such approaches and literature on MM
96 phytoremediation by using autochthonous plant species is becoming more important this last
97 decade (Ilunga wa Ilunga et al., 2015; Marrugo-Negrete et al., 2015; Ghazaryan et al., 2019).
98 However, fewer restoration trials are conducted in brownfields generating diffuse pollution of
99 soils. One of those brownfields, the Escalette site, is located on the Mediterranean coast of
100 Marseille (south-eastern France) at the outskirts of the city center. This site sheltered a lead-

101 smelting factory that operated from 1851 to 1925 using galena imported from Mediterranean
102 countries (Daumalin and Raveux, 2016). This activity contributed to a diffuse MM
103 contamination of surface soils of the surrounding area by slag deposits and by dispersion of
104 contaminated ashes from the factory horizontal chimney *via* erosion and bioalteration
105 processes (Testiati et al., 2013; Heckenroth et al., 2016a; Laffont-Schwob et al., 2016). The
106 brownfield is now included in the protected area of the Calanques National Park (PNCal), the
107 first French peri-urban National Park founded in 2012. This territory gathers several socio-
108 environmental issues (environmental health, biodiversity conservation and management,
109 regulatory issues) linked with anthropogenic disturbances (industrial and urban pollution, soil
110 erosion, over-frequentation), affecting this urban and natural protected and biodiversity-rich
111 area (Affre et al., 2015). At the Escalette site, the natural resilience capacity of the original
112 ecosystem has been strongly affected by MM soil contamination, which is currently reflected
113 by the presence of unvegetated scree which can constitute a source of contamination transfer
114 by water erosion and/or wind (Heckenroth et al., 2016b). In November 2015, a joint active
115 ecological restoration and phytostabilization trial has been implemented in this site
116 (Heckenroth et al., 2016a). This pilot-scale experiment aims at stabilizing MM soil
117 contamination sustainably by favoring a permanent native vegetation cover to limit its
118 transfer, while considering restoration of plant and soil biodiversity and functionalities. Three
119 years after the implementation of this experiment, two main questions remain: (i) At which
120 step along an ecological restoration trajectory correspond the implemented plant
121 communities? (ii) Do they ensure effective stabilization of MM soil contamination? To
122 answer these questions, a comparative *in situ* monitoring of soil properties, MM
123 concentrations in soils and in runoff water and characteristics of plant communities was
124 performed. These parameters were monitored over three *in situ* treatments: ecological
125 restoration plots characterized by the handling of physical environment variables (creation of
126 cultivation terraces) and plants, negative control plots without vegetation but with a physical
127 environment variable handling (terraces) such as ecological restoration plots, and positive
128 control plots with natural vegetation and nor abiotic neither biotic variables handling.

129

130 **2. Material and methods**

131

132 **2.1. Study site**

133 The study site is located in the Marseilleveyre calcareous massif, characterized by significant
134 karstic erosion and skeletal oligotrophic soils (Knoerr, 1959). The study site has a semi-arid

135 Mediterranean climate, characterized by hot and dry summers, mild and wet winters (Quézel
136 and Médail, 2003) and by a prevailing cold, dry and often violent wind, called Mistral
137 (Aillaud and Crouzet, 1988), accentuating the environment's aridity (average annual rainfall
138 of 500 mm, average annual temperature of 16 °C). The study site is located at the Escalette
139 industrial brownfield, at the outfall of the horizontal chimney of a former lead smelting
140 factory, in the protected area of the PNCal (WGS84 co-ordinates: 43.22578°N, 5.35059°E,
141 Fig. A). The site corresponds to a steep scree with an average slope of 23 %, located down
142 below a ridge at 140 m of altitude. This site is defined as a hotspot of contamination mainly
143 by lead (Pb) and arsenic (As) but also by copper (Cu) and zinc (Zn) (Testiati et al., 2013). The
144 soil, whose depth varies between 10 and 30 cm, corresponds to a mixture of limestone, clayey
145 limestone, marl (Knoerr, 1959) and contaminated ashes. A layer of ashes from a few to
146 several centimeters covers the soil at the outfall of the horizontal chimney, which can then be
147 defined as anthroposol.

148 In November 2015, an active ecological restoration has been carried out at the Escalette site
149 by environmental handling and planting. This operation is in accordance with the PNCal's
150 regulations, which prohibit the introduction of non-local plant material and the use of
151 amendments to conserve local biodiversity. 20 plots of 1 m² were created on the unvegetated
152 steep scree. These plots correspond to cultivation terraces made up of two strips, one
153 upstream and one downstream. Limestones present on the site were removed from the soil
154 surface and used to shape the terraces by installing a row of stones between upstream strip
155 and downstream strip of cultivation terraces. Limited soil handling was targeted. Eight plant
156 species from the local floristic background (constituted of calcareous xero-thermophilous
157 shrubland: Mediterranean scrubland, grasslands and stands of Aleppo pine) and defined as
158 pseudo-metallophytes according to the definitions given by Pauwels et al. (2006) and Faucon
159 et al. (2009) were transplanted in each plot: *Brachypodium retusum* (Pers.) P.Beauv., 1812,
160 *Cistus albidus* L., 1753, *Coronilla juncea* L., 1753, *Dianthus caryophyllus* L., 1753,
161 *Globularia alypum* L., 1753, *Lobularia maritima* (L.) Desv., 1815, *Rosmarinus officinalis* L.,
162 1753 and *Teucrium polium* L., 1753 (Heckenroth et al., 2017, Fig. B). Soil surface was
163 covered with limestones to limit water stress. A unique watering was done to the plant's earth
164 clod at the transplantation. Nor amendment adding neither watering was carried out after
165 transplantation.

166

167 **2.2. Experimental design**

168 Three treatments (E: ecological restoration plots, Cn: negative control plots, Cp: positive
169 control plots), each consisting of 5 replicates, were tested *in situ* (Fig. 1). E and Cn plots have
170 been located on the unvegetated scree at the beginning of the study and correspond to active
171 restoration with different levels of intervention. E plots are characterized by environmental
172 and plant handling (i.e. creation of cultivation terraces and planting, respectively). E plots
173 correspond to 5 plots selected from those implemented in November 2015 and contain planted
174 and spontaneous plant species. These E plots were selected for their well-established
175 perennial vegetation and the persistence of planted *C. juncea* individuals, assuming that their
176 highly developed root systems act on soil contamination fixation. Cn plots are characterized
177 by the same environmental handling as E plots, i.e. 1 m²-cultivation terraces located on the
178 unvegetated scree, but without planting. E and Cn plots were equipped with runoff water
179 collectors in November 2018. Runoff water collectors consist of a plastic chute placed at the
180 level of the downstream band of the plots, closed at the end and connected to a can of water
181 recovery by a funnel. A plastic tarpaulin was installed at ground level, under the stones of the
182 downstream band of the terraces and, attached to the chute. Another tarpaulin was placed over
183 the chute to recover only runoff water and not rainwater. This installed system can be easily
184 dismantled as required by the PNCal regulation. Cp plots correspond to the reference plant
185 community of this ecological restoration operation, i.e. a natural vegetation of young
186 Mediterranean scrubland that has sporadically persisted during past industrial activities until
187 today. No ecological restoration work was carried out on these plots, they have only been
188 spatially delimited by limestones. Thus, Cp plots have not been structured in cultivation
189 terraces or equipped with runoff water collectors to avoid disturbance and degradation due to
190 abiotic handling. Cp are located close to E and Cn plots to maintain comparable soil
191 contamination levels between the active restoration plots (E and Cn) and reference plots (Cp).
192 This allows a realistic ecological restoration objective to be defined on MM contaminated
193 soil.

194

195 **2.3. Soil analysis**

196 Soil samples were collected in 4 points at each plot corner in the top 15 cm-layer and pooled
197 (mix of an equal weight of the 4 subsamples on each plot). The obtained composite soil
198 samples for each E, Cp, and Cn plots were sieved at 2 mm and analyses of agronomic
199 characteristics, MM contamination and microbial properties were performed.

200

201 **2.3.1. Soil agronomic characteristics**

202 Soil agronomic analyses were carried out on 40 °C dried soil. pH was determined with the
203 Orion 2 Star Thermo Scientific SM30B device, in a solution of soil and distilled water (1/5,
204 V/V, NF ISO10390, 2005). Total nitrogen content (TKN) was estimated by the Kjeldahl
205 method (NF ISO 11261, 1995). Total organic carbon (TOC) was determined by the difference
206 between total carbon content and inorganic carbon content (NF ISO 10694, 1995).
207 Granulometry and texture (NF X 31-107, 2003), available phosphorus (P, NF ISO 11263,
208 1995) and cation exchange capacity (CEC, NF X 31-130, 1999) measurements were
209 performed in the Laboratoire Développement Méditerranée (Alès, France).

210

211 **2.3.2. Soil contamination**

212 Soil samples were dried at 40 °C and ground at 0.2 mm (RETSCH zm 1000 with tungsten
213 blades and titanium sieve) in order to evaluate soil contamination level and MM mobilization
214 in soil. In order to determine pseudo-total MM concentrations (MM_{tot}), samples were digested
215 in a microwave mineralizer (Milestone Start D) using aqua regia (1/3 HNO_3 + 2/3 HCl), then
216 filtered through a 0.45 μm cellulose ester membrane filter (NF ISO 11466, 1995). For mobile
217 MM concentrations (MM_{mob}), 0.05 M EDTA (pH 7.0 ± 0.1) solution was used as extractant
218 following the method approved by the Community Bureau of Reference (CBR)
219 (Quevauviller, 1998). A ratio of soil/EDTA solution corresponding to 1/10 w/V was used.
220 The solution was stirred at room temperature for 1 h, then centrifuged during 10 min at
221 8,000 rpm and filtered at 0.45 μm . MM_{tot} and MM_{mob} concentrations were determined by ICP-
222 AES (Jobin Yvon Horiba, Spectra 2000) for As, Cu, Pb and Zn. Quality controls and accuracy
223 were checked using standard soil reference materials (CRM 049-050, from RTC-USA), with
224 accuracies within 100 ± 10 %. Contamination factors (CF) were calculated according to the
225 formula:

226

227 Eq. 1. $CF_{MM} = [MM]_{tot} / [MM]_{local\ background\ value}$, where $[MM]_{local\ background\ value}$ are 4.9, 7.5, 42.9,
228 3.1 and 66 $mg.kg^{-1}$ for As, Cu, Pb, Sb and Zn, respectively, as previously reported with the
229 same method used in the present study (Affholder et al., 2014).

230

231 Then, the multi-contamination level was estimated by calculating the pollution load index
232 (PLI) according to the formula (Rashed, 2010; Affholder et al., 2014):

233

234 Eq. 2. $PLI = \sqrt[4]{CF_{As} \times CF_{Cu} \times CF_{Pb} \times CF_{Zn}}$

235

236 Percentage of mobile MM in soil were calculated according to the formula (Affholder, 2013):

237

238 Eq. 3. $\%MM_{\text{mob}} = ([MM]_{\text{mob}}/[MM]_{\text{tot}}) \times 100$

239

240 **2.3.3. Soil microbial analysis**

241 Soil microbial analyses were performed in order to assess the eco-physiological state of soil
242 microbial communities.

243 Soil basal respiration (SBR) and substrate-induced respiration (SIR) were measured to assess
244 global microbial activity and biomass (Anderson and Domsch, 1978; Anderson, 2003). Ten
245 grams dry weight equivalent of fresh soil at 30 % of water holding capacity were placed in
246 117 mL slightly open ajar glass jars and incubated for 48 h at 23 °C. For SBR measurement,
247 each sample was oxygenated for 4 min and incubated for 4 h at 23 °C in tightly closed glass
248 jars. Then, 1 mL of their atmosphere was sampled in the headspace with a syringe and
249 injected into a gas chromatograph (Chrompack CHROM 3 – CP 9001) to analyse CO₂
250 production by microbial oxidation of the soil organic matter. SIR was estimated using a
251 procedure from Anderson and Domsch (1978). Soil samples were amended with 450 mg of
252 talc and 50 mg of powdered anhydrous glucose to maximize the respiration rate and incubated
253 for 2 h at 23 °C in slightly open ajar glass jars. Then, each sample was oxygenated for 4 min
254 and then incubated for 2 h at 23 °C in tightly closed glass jars. Then, 1 mL of their
255 atmosphere was sampled in the head space with a syringe and injected into a gas
256 chromatograph (Chrompack CHROM 3 – CP 9001) to analyse CO₂ production by microbial
257 oxidation of glucose. The gas chromatograph was equipped with a thermal conductivity
258 detector and a packed column (Porapack). The carrier gas helium flow was regulated at
259 60 mL.h⁻¹. Ambient CO₂ concentrations were subtracted from sampled CO₂ concentrations
260 and resulting values were adjusted at 22 °C according to Ideal Gas Laws using a Q10 = 2. SIR
261 was converted into microbial biomass (MB) using the relation established by Beare et al.
262 (1990). Then, the metabolic quotient (qCO₂), which is a sensitive eco-physiological indicator
263 of soil stress induced by environmental conditions (Anderson, 2003), was obtained by
264 calculating the SBR/MB ratio.

265 Glomalin-related soil protein (GRSP), a fungal glycoprotein synthesized by certain arbuscular
266 mycorrhizal fungi involved in soil quality (Zhu and Miller, 2003; Rillig et al., 2004) and MM
267 chelation (Cornejo et al., 2008; Vodnik et al., 2008), was quantified. Three analytical
268 replicates were made for each sample. GRSP was extracted by repeated autoclaving
269 extraction (Advantage-Lab ALO2-05) from 0.5 g of 40 °C dried soil in 4 mL of 50 mM

270 citrate pH 8.0 at 121 °C for 60 min. After each autoclaving cycle, the supernatant was
271 removed by centrifugation (Hettich Zentrifugen Universal 320R) at 7000 g for 20 min. The
272 extraction cycles were repeated until the supernatant had lost its dark brown-red color (8
273 cycles). Supernatants of the different extraction cycles of the same analytical replica were
274 pooled and then centrifuged at 7000 g for 10 min. GRSP content was measured in 1 mL
275 supernatant by spectrophotometric dosage (595 nm) according to Bradford method, by using a
276 Bovin Serum Albumin solution as standard. Finally, GRSP concentration was converted to
277 g.kg^{-1} of dry soil.

278

279 **2.4. Runoff water analysis**

280 Runoff water were collected on each E and Cn plots over the period from 24 January 2019 to
281 15 April 2019 during which there was 97.75 mm of rainfall. MM concentrations in runoff
282 water (MM_{water}) were determined by ICP-AES (Jobin Yvon Horiba, Spectra 2000) for As, Cu,
283 Pb and Zn, for each E and Cn plot in order to evaluate MM transfer by hydric erosion. Before
284 that, 20 mL of runoff water were filtered to 0.45 μm , and 0.2 mL of nitric acid (Trace metal
285 grade HNO_3) were added to them. Quantity of each MM exported in runoff water (MM_{water})
286 was calculated according to the formula:

287

288 Eq. 4. $\text{MM}_{\text{water}} = [\text{MM}]_{\text{water}} / \text{water volume of the collector}$

289

290 **2.5. Vegetation analysis**

291 Vegetation analysis was performed in order to characterize the structure (plant cover), the
292 composition (richness, diversity, equitability) and the sustainability (Raunkier plant life-form,
293 Grime adaptative strategy) of each plant community. Cn plots, which were unvegetated at the
294 beginning of this study, were considered in plant community analysis because plants
295 established during the experiment.

296 Plant cover was estimated by different percentage class: < 10 %, 10-25 %, 25-50 %, 50-75 %,
297 > 75 % (Braun-Blanquet et al., 1952). The following indices were estimated along with
298 presence/absence and abundance field data: specific richness, Shannon's diversity index, and
299 Pielou's equitability index (Wheater et al., 2011).

300 Plant functional traits (Garnier and Navas, 2013; Pérez-Harguindeguy et al., 2016) were
301 studied from BASECO database (Gachet et al., 2005). Percentages of plant species in a plot
302 per Raunkier plant life-form (geophyte, chamaephyte, hemicryptophyte, therophyte,
303 phanerophyte) correspond to the ratio of the number of species in a plot with a given Raunkier

304 life-form on the total number of species in a plot. Percentages of plant species in a plot per
305 Grime adaptative strategy (stress tolerant, competitive stress tolerant, ruderal stress tolerant,
306 competitive ruderal stress tolerant) correspond to the ratio of the number of species in a plot
307 with a given Grime adaptative strategy on the total number of species in a plot.

308

309 **2.6. Statistical analysis**

310 Statistical analyses were conducted with R (3.4.2 version). *P*-values were considered as
311 significant when they were less than or equal to 0.05.

312 The two plant categories in E plots (i.e. planted and spontaneous) have been considered
313 without distinction in statistical analysis because it was not possible to discriminate on field
314 planted individuals from spontaneous individuals which came from vegetative propagation or
315 seedlings from seeds of planted individuals or from the soil seed bank.

316 Kruskal-Wallis tests, followed by Dunn's post-hoc tests, were carried out to study the impact
317 of experimental treatment (Cn, E, Cp) on soil (physicochemical and microbial) and plant
318 community characteristics. Mann-Whitney tests were performed to study the impact of
319 experimental treatment (Cn, E) on MM transfer in runoff water.

320 A path analysis was performed to evaluate the impacts of MM soil contamination on
321 ecological restoration dynamic and the impacts of vegetal succession on agronomic and
322 microbial soil quality. Path analysis is a particular case of structural equation modelling
323 (SEM). Its permits to represent causal networks between several measured variables and to
324 test model data consistency (Grace, 2006). Path analysis was performed by using the *lavaan*
325 package (Rosseel, 2012). A conceptual model was developed from *a priori* knowledge
326 consistent with our data and having biological sense (Fig. C). Five groups of measured
327 variables were created by using axis 1 coordinates of principal component analysis (PCA):
328 MM soil contamination ($[As]_{tot}$, $[Cu]_{tot}$, $[Pb]_{tot}$, $[Zn]_{tot}$; PCA axis 1 explaining 84.5 % of the
329 variation), MM mobilization in soil and transfer by water erosion ($[As]_{mob}$, $[Cu]_{mob}$, $[Pb]_{mob}$,
330 $[Zn]_{mob}$, $[As]_{H_2O}$, $[Cu]_{H_2O}$, $[Pb]_{H_2O}$, $[Zn]_{H_2O}$; PCA axis 1 explaining 49.6 % of the variation),
331 agronomic soil quality (pH, TOC, TKN, C/N, P, CEC, clay percentage; PCA axis 1
332 explaining 55.9 % of the variation), microbial activities and biomass (SBR, MB, GRSP; PCA
333 axis 1 explaining 88.2 % of the variation), plant community parameters (plant cover, specific
334 richness, Shannon's diversity index, Pielou's equitability index, chamaephytes,
335 hemicryptophytes and competitive stress tolerant species; PCA axis 1 explaining 74.2 % of
336 the variation). The full model was simplified by stepwise exclusion of non-significant
337 variables until a minimal adequate model was reached. The adequacy of the model was

338 determined by non-significant differences between predicted and observed covariance
339 matrices (chisquared test, $P > 0.05$), a low Root Mean Squared Error (RMSEA < 0.10), a high
340 Comparative Fit Index (CFI > 0.90) and a low Standardized Root Mean Square Residual
341 (SRMR < 0.08) (Grace, 2006; Rosseel, 2012). The selected model does not consider MM
342 mobilization in soil and transfer by hydric erosion because these variables do not permit to
343 obtain an adequate model. Thus, Spearman correlation tests (correlation between two
344 variables) were performed in order to test the relationship between MM mobilization in soil
345 and transfer by hydric erosion and MM soil contamination, agronomic soil quality, microbial
346 activities and biomass, plant cover, diversity and sustainability.

347

348 **3. Results**

349 **3.1. Soil physicochemical characteristics**

350 Six parameters were significantly affected by the experimental treatment (Tables 1 and A).
351 pH was 11 % higher for Cn than for Cp plots (Table 1). TOC content was 3.9 and 1.9 times
352 higher in Cp than in Cn and E plots, respectively (Fig. 2a, Table 1). CEC was 2.1 and 1.4
353 times higher in Cp than in Cn and E plots, respectively (Fig. 2b, Table 1). TKN was 2 times
354 higher in Cp and E than in Cn plots (Fig. 2c, Table 1). As_{mob} percentage was 2.1 times higher
355 in Cp than in Cn plots (Table 1). Zn_{mob} percentage was respectively 1.7 and 1.4 times higher
356 in Cp and E than in Cn plots (Table 1).

357

358 **3.2. Soil microbial properties**

359 Two parameters were significantly affected by the experimental treatments (Tables 1 and A).
360 SBR was 4.2 and 5.2 times higher in Cp than in Cn and E plots, respectively (Table 1). GRSP
361 content was 2.8 and 2.1 times higher in Cp than in Cn and E plots, respectively (Fig. 2d,
362 Table 1).

363

364 **3.3. Vegetation characteristics**

365 Ten parameters were significantly affected by the experimental treatments (Tables 1 and A).
366 Plant cover was respectively 40.0 and 21.7 times higher in Cp and E plots than in Cn plots
367 (Fig. 3a); specific richness was respectively 6.7 and 9.0 times higher in Cp and E plots than in
368 Cn plots; Shannon's diversity index was respectively 8.3 and 9.6 times higher in Cp and E
369 plots than in Cn plots (Fig. 3b); Pielou's equitability index was respectively 26.3 and 25.0
370 times higher in Cp and E plots than for Cn plots; percentage of hemicryptophyte species
371 respectively 18.1 and 9.0 times higher in Cp and E plots than for Cn plots (Fig. 3c);

372 percentage of competitive stress tolerant species respectively 83.3 and 29.8 times higher in Cp
373 and E plots than for Cn plots (Fig. 3d); percentage of chamaephyte species respectively 28.7
374 and 25.0 times higher in Cp and E plots than for Cn plots (Table 1). Cp plots presented a
375 percentage of competitive stress tolerant species 2.8 times higher than in E plots (Fig. 3d,
376 Table 1). E and Cn plots presented a percentage of therophyte species respectively 16.7 and
377 14.7 times higher than in Cp plots (Fig. 3e, Table 1). E plots had a percentage of ruderal stress
378 tolerant species 3.3 and 7.0 times higher than in Cp and Cn plots, respectively (Fig. 3f, Table
379 1). In E plots, most of the transplanted native plant species remained and other spontaneous
380 plant species appeared such as *Sonchus bulbosus* (L.) N. Kilian & Greuter, 2003, *Linaria*
381 *supina* (L.) Chaz., 1790 or *Centranthus calcitrapae* (L.) Dufr., 1811. Spontaneous plant
382 species also appeared in Cn plots (Table B).

383

384 **3.4. Correlations between soil, runoff water and plant community characteristics**

385 Path analysis (Fig. 4) showed that MM soil contamination negatively affected vegetation
386 characteristics (i.e. plant cover, diversity and sustainability) and agronomic soil quality.
387 Vegetation characteristics positively affected agronomic soil quality. Agronomic soil quality
388 positively affected soil microbial properties (i.e. microbial biomass and activities). MM soil
389 contamination and vegetation characteristics showed indirect effects on soil microbial
390 properties mediated by degradation and improvement of agronomic soil quality, respectively.
391 Spearman correlation tests showed that MM soil contamination was positively correlated to
392 MM mobilization in soil and transfer by hydric erosion ($r = 0.89$, $P < 0.001$).

393

394 **4. Discussion**

395 The aim of this study was to evaluate the evolution of the ecological restoration trajectory and
396 the effectiveness of phytostabilization at the peri-urban and protected site of the Escalette by a
397 comparative *in situ* monitoring of soil properties, MM concentrations in soils and in runoff
398 water and, characteristics of plant communities.

399

400 **4.1. Evaluation of ecological restoration success after three years**

401 Three attributes are mainly used to evaluate the success of an ecological restoration operation
402 (Ruiz-Jaen and Mitchell Aide, 2005) out of the nine defined by the Society of Ecological
403 Restoration (SER, 2016): diversity of the organism communities (richness, abundance),
404 vegetation structure (cover, height, biomass) and ecological processes (biotic interactions,

405 nutrient and organic matter cycles). Compared to the reference plant community (i.e. a young
406 Mediterranean scrubland for the present study), these features are milestones enabling to
407 assess the ecosystem position on the restoration trajectory. When ecosystem disturbances are
408 due to industrial pollution such as in this study site, the level of soil contamination is also an
409 important parameter to consider in assessing the environmental physical conditions because it
410 affects the composition, structure and functioning of the ecosystem (Wong, 2003; Li, 2006).

411 At the Escalette site, plant communities in the reference plots (Cp plots, young Mediterranean
412 scrubland) and in the ecological restoration plots (E plots) had similar composition, relatively
413 low diversity and high equitability during the study. However, they differed by their plant
414 community structure and functions. In the reference plots, plant communities formed a
415 completely permanent vegetation cover mainly shaped by hemicryptophytes and
416 chamaephytes with competitive stress tolerant strategy. However, only a partial permanent
417 plant cover occurred in ecological restoration plots (planted and spontaneous
418 hemicryptophytes and chamaephytes with competitive stress tolerant strategy) with also
419 annual species forming a more or less temporary vegetation cover (therophytes with ruderal
420 and tolerant stress strategies) (Garnier and Navas, 2013; Pérez-Harguindeguy et al., 2016).
421 Establishment of a dense and permanent vegetation cover is a central element for the success
422 of an ecological restoration operation (Cortina et al., 2011). Indeed, this allows the creation of
423 favorable micro-niches to the establishment of new local plant species by improving
424 environmental conditions, notably edaphic ones, thus favoring plant succession and soil
425 microbial functions (Padilla and Pugnaire, 2006; Padilla et al., 2009). At the Escalette site, the
426 establishment of a large, diversified and sustainable plant cover is negatively affected by soil
427 MM contamination. Indeed, soil contamination can constrain plant establishment and
428 persistence because MM may cause toxic, oxidative and/or hydric stresses on plants leading
429 to disruption of physiological processes (i.e. respiration, photosynthesis, water and nutrients
430 absorption) essential for plant growth and development (Baryla et al., 2001; Yadav, 2010;
431 Michalak, 2016). Moreover, MM contamination adds to intrinsic abiotic stress (i.e. scarce and
432 scattered rain events, high summer temperatures, wind) which can strongly constraint plant
433 establishment, growth and development (Bolan et al., 2011; Kumar Yadav et al., 2018).

434

435 Soil quality is also an important parameter to consider in an ecological restoration operation
436 because soil functioning recovery is essential to pass the resilience threshold (Heneghan et al.,
437 2008). Overall, the Escalette soils were characterized by poor agronomic quality (low organic
438 matter, nutrient contents, high C/N ratio) (Thomas et al., 2006; Gobat et al., 2013). The high

439 MM soil contamination in this site negatively impacted the agronomic soil quality. A
440 competition for adsorption onto soil particles can occur between toxic MM and essential
441 cations for plant development (Adriano et al., 2004; Bolan et al., 2011; Kumar Yadav et al.,
442 2018). This competition is governed by physicochemical soil parameters (MM concentrations,
443 redox potential, cation exchange capacity, organic matter content and in particular pH) which
444 influence MM distribution between solid soil phase and soil solution thus affecting MM
445 mobility and bioavailability (Adriano et al., 2004; Bolan et al., 2011). Moreover, microbial
446 communities were submitted to significant stress (high metabolic quotient whatever the
447 treatment) due to the indirect negative effect of high MM contamination on microbial
448 properties mediated by degradation of soil quality. This resulted in low microbial biomass as
449 previously reported by Kızılkaya et al. (2004) and Gülser and Erdoğan (2008). This low
450 biomass can be explained by a metabolic quotient increase. This indicates that sensitive
451 microorganisms decrease whereas resistant microorganisms increase their respiration activity
452 to maintain their basal metabolism at the expense of biosynthesis, resulting in their biomass
453 decrease (Fließbach et al., 1994; Friedlová, 2010).

454

455 However, plant community differences between the reference plots, the ecological restoration
456 plots and the unvegetated plots (Cn plots) induced soil properties variations. The completely
457 permanent vegetation cover in the reference plots improved agronomic soil quality and
458 therefore soil microbial activities and biomass compared to the ecological restoration and
459 unvegetated plots. This may be due to regular litter input resulting in soil enrichment in
460 organic matter and nutrients which increase microbial activities and biomass as reported by
461 Kızılkaya et al. (2004), Gülser and Erdoğan (2008) and Friedlová (2010). Among these
462 microbial activities, glomalin secretion by arbuscular mycorrhizal fungi was higher in the
463 reference plots compared to the ecological restoration and unvegetated plots. This
464 glycoprotein is known to enhance soil organic matter content (Rillig et al., 2001; Zhu and
465 Miller, 2003), soil aggregation and structural stability (Rillig, 2004). In the ecological
466 restoration plots, soil nitrogen content was higher than in the unvegetated ones. The
467 occurrence of *C. juncea* individuals in the ecological restoration plots, a leguminous engineer
468 plant in association with nitrogen-fixing bacteria and endomycorrhizal fungi (Carrasco et al.,
469 2011), may facilitate other plant establishment by enhancing soil quality in particular nitrogen
470 content (Padilla and Pugnaire, 2006).

471

472 Results of this study suggest that an ecological restoration trajectory was initiated at the
473 ecological restoration plots (Fig. 5) according to the floristic composition and vegetation
474 analysis processed in the Calanques National Park by Heckenroth et al. (2016b). Several
475 hypotheses on ecological restoration trajectory evolution can be emitted. On the one hand,
476 similarities of plant communities between the reference and the ecological restoration plots
477 and presence of a partial annual plant cover at the ecological restoration plots may indicate
478 that these plant communities are at an earlier stage of vegetation succession relative to the
479 reference plots (*sensu* Aronson et al., 1993). Thus, ecological restoration trajectory may tend
480 towards reference state, i.e. a young Mediterranean scrubland. On the other hand, differences
481 in ecological niches between the reference plots and the ecological restoration plots (MM
482 contamination heterogeneity, the occurrence of Aleppo pines close to Cp plots creating
483 shading and litter inputs) can lead to the establishment of alternative stable states at the
484 ecological restoration plots. Indeed, during an ecological restoration operation in
485 Mediterranean area with limited human intervention, return to the reference state is rarely
486 reached because aridity may keep the ecosystem below the irreversibility threshold (Aronson,
487 1993; Young et al., 2001, 2005).

488

489 **4.2. Evaluation of phytostabilization success after three years**

490 MM mobilization in soil and MM transfer by water erosion were not significantly different
491 between the unvegetated and the ecological restoration plots. It was therefore not possible to
492 demonstrate the stabilizing effect of soil contamination by plant communities and their
493 associated microorganisms. This may be due to the temporary and removable runoff water
494 collectors which were not really adapted to the scarce and scattered rain events which
495 occurred during the study period. However, this system implementation was restricted by
496 PNCal regulations in this protected area. Most of the studies used microcosms equipped with
497 a downstream water collector to recover only runoff water from the plot (Bochet et al., 1998;
498 Zhang et al., 2004; Martínez Raya et al., 2006). But, in this study, plots were not isolated from
499 the local ecosystem. Thus, the runoff water recovered in collectors came from the plots
500 themselves but also may come from upstream areas, generating a response more at the level
501 of the ecosystem watershed than the plots.

502

503 After three years, no significant effect of soil agronomic quality, microbial and plant
504 communities on MM immobilization and transfer were detected, although the literature
505 indicates that improvement of soil quality, microbial functionalities, plant cover and

506 sustainability allow enhancing MM immobilization (Mendez et al., 2007; Mendez and Maier,
507 2008; Valentín- Vargas et al., 2014). This result may be due to several parameters. Firstly, in
508 this study, improvement of soil physicochemical properties was only related to the action of
509 engineering native plant species and associated microorganisms, contrary to most
510 phytostabilization studies using amendments (Rizzi et al., 2004; Radziemska et al., 2018).
511 Thus, at the ecological restoration plots, improving the soil quality exclusively by plants and
512 their impact on MM retention may take more time than when using amendments which hasten
513 MM toxicity reduction and limit their bioavailability by adsorption and complexation
514 processes (Rizzi et al., 2004; Mendez and Maier, 2008; Epelde et al., 2009; Radziemska et al.,
515 2018). Then, MM immobilization by microorganisms by biosorption, bioaccumulation and
516 transformation (Adriano et al., 2004; Khan, 2005; Ma et al., 2011) is limited by low biomass
517 and microbial activities at the ecological restoration plots. Finally, the temporary vegetation
518 cover of annual species with shallow roots may have not allowed a sustainable stabilization of
519 MM contamination at the ecological restoration plots. Phytostabilization is enhanced by
520 persistent and dense root systems and plant cover that reduce soil erosion by decreasing
521 runoff water, protecting soil surface, increasing water infiltration and soil quality (Bochet et
522 al., 1998; 2006; Durán Zuazo et al., 2006; 2008; Bolan et al., 2011) and immobilizing MM in
523 roots and/or rhizosphere (Adriano et al., 2004; Pilon-Smits, 2005; Bolan et al., 2011).
524 Therefore, without amendment inputs and under drastic Mediterranean environmental
525 conditions, native plant communities and their associated microorganisms may need more
526 time before improving MM stabilization by enhancing physicochemical soil parameters
527 involved in MM retention and by biological mechanisms. Thus, a longer observation period
528 may be necessary to conclude on a potential phytostabilization effect of these plant
529 communities. It would also be interesting to take into account other aspects of soil microbial
530 communities (litter decomposition, C and N mineralization, functional diversity) which are
531 driving parameters for good management of soil MM contamination (Brookes, 1995;
532 Friedlová, 2010, Colin et al., 2019).

533

534 **5. Conclusions**

535 The objectives of this study were to evaluate the evolution of restoration trajectory at the
536 ecological restoration plots and the effectiveness of soil MM stabilization *via* soil, runoff
537 water and plant community analyses. Three years after the implementation of the ecological
538 restoration plots at the Escalette site, it appears that the mobilized ecological engineering tools
539 have enabled the establishment of perennial and annual plant communities at these plots.

540 However, there is no evidence of a significant improvement of soil quality and
541 phytostabilization efficiency. This may be due to the non-use of amendments and a too short
542 observation period.

543 The active restoration actions carried out in this Mediterranean peri-urban and MM
544 contaminated area, based on abiotic (creation of cultivation terraces) and biotic (native plant
545 species planting) filter handling by less intrusive techniques on the environment adequate
546 with regulations of a protected area, seem to allow the creation of micro-niches favorable to
547 the establishment of resilient dynamics of the native plant communities. The obtained results
548 support the fact that it is preferable to intervene in these environments rather than to leave in
549 place unvegetated screes with risks of erosion of contaminated soil particles and transfer to
550 the food web.

551

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566

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816

817 **Figure captions**

818

819 **Figure 1.** Location of (a) the Escalette site in the Calanques National Park (modified after
820 Heckenroth et al., 2016b), (b) the Escalette lead smelting factory, horizontal chimney and
821 study area (source: Google Earth), (c) Cn plots (red, negative control), E plots (yellow,
822 ecological restoration plot) and Cp plots (green, positive control) plots in the study area
823 (source: Géoportail) and (d) characteristics of Cn, E and Cp plots.

824

825 **Figure 2.** Total organic carbon (TOC) concentration, total Kjeldahl nitrogen (TKN)
826 concentration, cation exchange capacity (CEC) and glomalin-related soil protein (GRSP)
827 concentration in Cn plots (negative control, n = 5), E plots (ecological restoration plots, n = 5)
828 and Cp plots (positive control, n = 5). Different letters above boxplots indicate a significant
829 difference between plots for a given parameter ($P \leq 0.05$).

830

831 **Figure 3.** Plant cover, Shannon's diversity index, percentage of hemicryptophyte species,
832 percentage of therophyte species, percentage of competitive stress tolerant species and
833 percentage of ruderal stress tolerant species in Cn plots (negative control, n = 5), E plots
834 (ecological restoration plots, n = 5) and Cp plots (positive control, n = 5). Different letters
835 above box plots indicate a significant difference between plots for a given parameter
836 ($P \leq 0.05$).

837

838 **Figure 4.** Path analysis performed between agronomic soil quality, MM soil contamination,
839 microbial activities and biomass, plant cover, diversity and sustainability. The arrows
840 represent the relationships between the different parameters. Red arrow indicates a negative
841 relationship and green arrow a positive relationship. The thicker is the arrow, the greater is the
842 correlation. Correlation coefficients and significance levels of the correlations (*: $P \leq 0.05$;
843 **: $P \leq 0.01$; ***: $P \leq 0.001$) are indicated at each arrow.

844

845 **Figure 5.** Ecological restoration process and perspectives after three years at the Escalette
846 site.

847

848 **Table captions**

849 **Table 1.** Averages \pm standard errors ($n = 5$) of soil (TOC: total organic carbon, TKN:
850 Kjeldahl nitrogen, CEC: cation exchange capacity, $[As]_{tot}$, $[Cu]_{tot}$, $[Pb]_{tot}$, $[Zn]_{tot}$: arsenic,
851 copper, lead and zinc pseudo-total concentrations in soil, PLI: pollution load index, $[As]_{mob}$,
852 $[Cu]_{mob}$, $[Pb]_{mob}$, $[Zn]_{mob}$: arsenic, copper, lead and zinc mobile fraction concentrations in soil,
853 and % (in brackets) of mobile versus pseudo-total MM in soil, SBR: soil basal respiration,
854 MB: microbial biomass, qCO_2 : metabolic quotient, GRSP: glomalin-related soil protein),
855 runoff water (As_{water} , Cu_{water} , Pb_{water} , Zn_{water} : quantities of arsenic, copper, lead and zinc
856 exported in runoff water) and plant communities parameters of Cn plots (negative control,
857 $n = 5$), E plots (ecological restoration plots, $n = 5$) and Cp plots (positive control, $n = 5$).
858 Different letters indicate a significant difference between plots for a given parameter
859 ($P \leq 0.05$).

860

861

862

863 **Appendix captions**

864

865 **Figure A.** Localization of the Escalette study site in Marseilleveyre massif (Calanques
866 National Park).

867

868 **Figure B.** Schematic representation of Escalette's E plots organized in two terraces and
869 planted with eight pseudo-metallophytes species from the local floristic background in
870 November 2015 (modified from Heckenroth, 2017).

871

872 **Figure C.** Conceptual model of path analysis taking into account agronomic soil quality, MM
873 soil contamination, microbial activities and biomass, plant cover, diversity and sustainability.
874 The arrows represent the relationships between the different parameters. Red arrow indicates
875 a negative relationship and green arrow a positive relationship.

876 **1** – MM soil contamination impacts negatively plant cover, diversity and sustainability. MM
877 soil contamination may cause oxidative and hydric stresses disturbing essential physiological
878 process of plant growth and development (respiration, photosynthesis, water and nutrients
879 absorption) (Baryla et al., 2001; Yadav, 2010; Michalak, 2016).

880 **2** – MM soil contamination impacts negatively agronomic soil quality. MM soil
881 contamination potentially created a competition between essential cations for plant
882 development and toxic MM for adsorption to soil particles (Bolan et al., 2011).

883 **3** – MM soil contamination impacts negatively microbial activities and biomass. MM soil
884 contamination may cause a reduction of microbial number, diversity and activities (Fließbach
885 et al., 1994; Brookes, 1995; Kızılkaya et al., 2004; Gülser et Erdoğan, 2008).

886 **4** – Increase in plant cover, diversity and sustainability impacts positively agronomic soil
887 quality. A permanent, dense and diversified plant cover improves soil organic matter, nitrogen
888 and nutrient contents by regular litter inputs (Gobat et al., 2013).

889 **5** – Improvement of agronomic soil quality, in particular organic matter quantity and quality,
890 impacts positively microbial activities and biomass (Kızılkaya et al., 2004; Gülser et Erdoğan,
891 2008; Friedlová, 2010; Gobat et al., 2013).

892 **6** – The higher the MM soil contamination is, the more MM are mobilizable and transferable
893 (Bolan et al., 2011).

894 **7, 8, 9** - Improvement of soil quality, microbial functionalities and plant cover, diversity and
895 sustainability allows to enhance MM immobilization by physicochemical and biological

896 mechanisms. This reduces MM mobilization in soil and transfer by hydric erosion (Adriano et
897 al., 2004; Pilon-Smits, 2005; Bolan et al., 2011).

898

899 The adequacy of the full model was not good (P of $\chi^2 = 0.020$). Thus, we removed non-
900 significant relationships (P -value of relationship 3 = 0.656; P -value of relationship 7 = 0.864;
901 P -value of relationship 8 = 0.306; P -value of relationship 9 = 0.696) from this model.
902 Exclusion of non-significant relationships did not permit to obtain a good adequacy of the
903 new model (P of $\chi^2 = 0.010$, CFI = 0.850, SRMR = 0.111). Finally, by removing the
904 relationship 6, we obtained an adequate model (p of $\chi^2 = 0.061$, RMSEA = 0.067, CFI = 0.904
905 and SRMR = 0.063) and this final model was selected.

906

907 **Table A.** (a) P -values of Kruskal-Wallis and Dunn tests performed for soil characteristics
908 (TOC: total organic carbon, TKN: Kjeldahl nitrogen, CEC: cation exchange capacity, $[\text{As}]_{\text{tot}}$,
909 $[\text{Cu}]_{\text{tot}}$, $[\text{Pb}]_{\text{tot}}$, $[\text{Zn}]_{\text{tot}}$: arsenic, copper, lead and zinc pseudo-total concentrations in soil, PLI:
910 pollution load index, $\text{As}_{\text{mob}}(\%)$, $\text{Cu}_{\text{mob}}(\%)$, $\text{Pb}_{\text{mob}}(\%)$, $\text{Zn}_{\text{mob}}(\%)$: percentages of mobile
911 arsenic, copper, lead and zinc in soil, SBR: soil basal respiration, MB: microbial biomass,
912 $q\text{CO}_2$: metabolic quotient, GRSP: glomalin-related soil protein) and plant communities
913 parameters of Cn plots (negative control, $n = 5$), E plots (ecological restoration plots, $n = 5$)
914 and Cp plots (positive control, $n = 5$), (b) P -values of Mann-Whitney tests performed for
915 runoff water parameters (As_{water} , Cu_{water} , Pb_{water} , Zn_{water} : quantities of arsenic, copper, lead and
916 zinc exported in runoff water) of Cn and E plots. Significant difference between treatments
917 are indicated by stars (*: $P \leq 0.05$; **: $P \leq 0.01$; ***: $P \leq 0.001$).

918

919 **Table B.** Floristic inventory on E plots (ecological restoration plots), Cp plots (positive
920 control) and Cn plots (negative control).

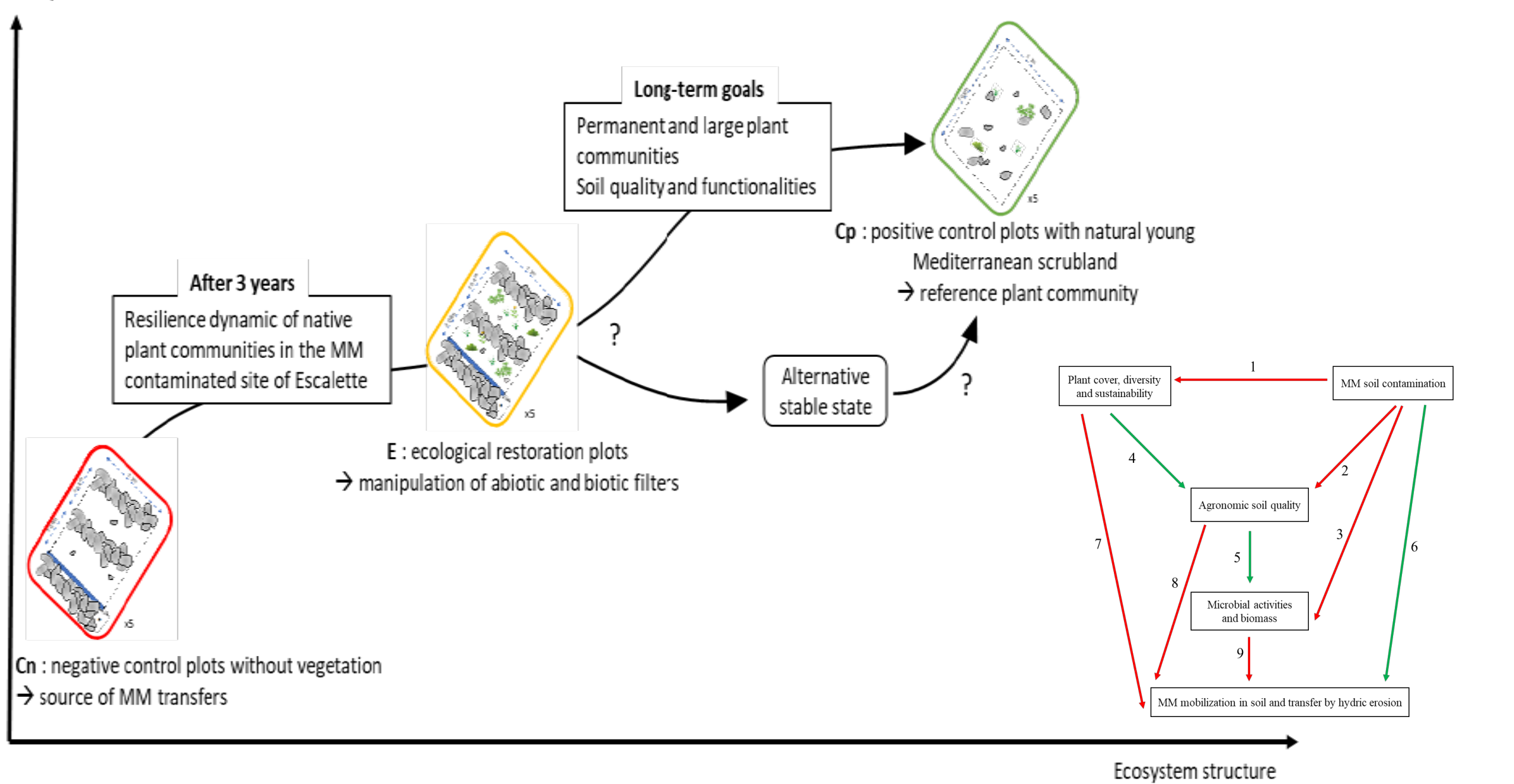


Figure1

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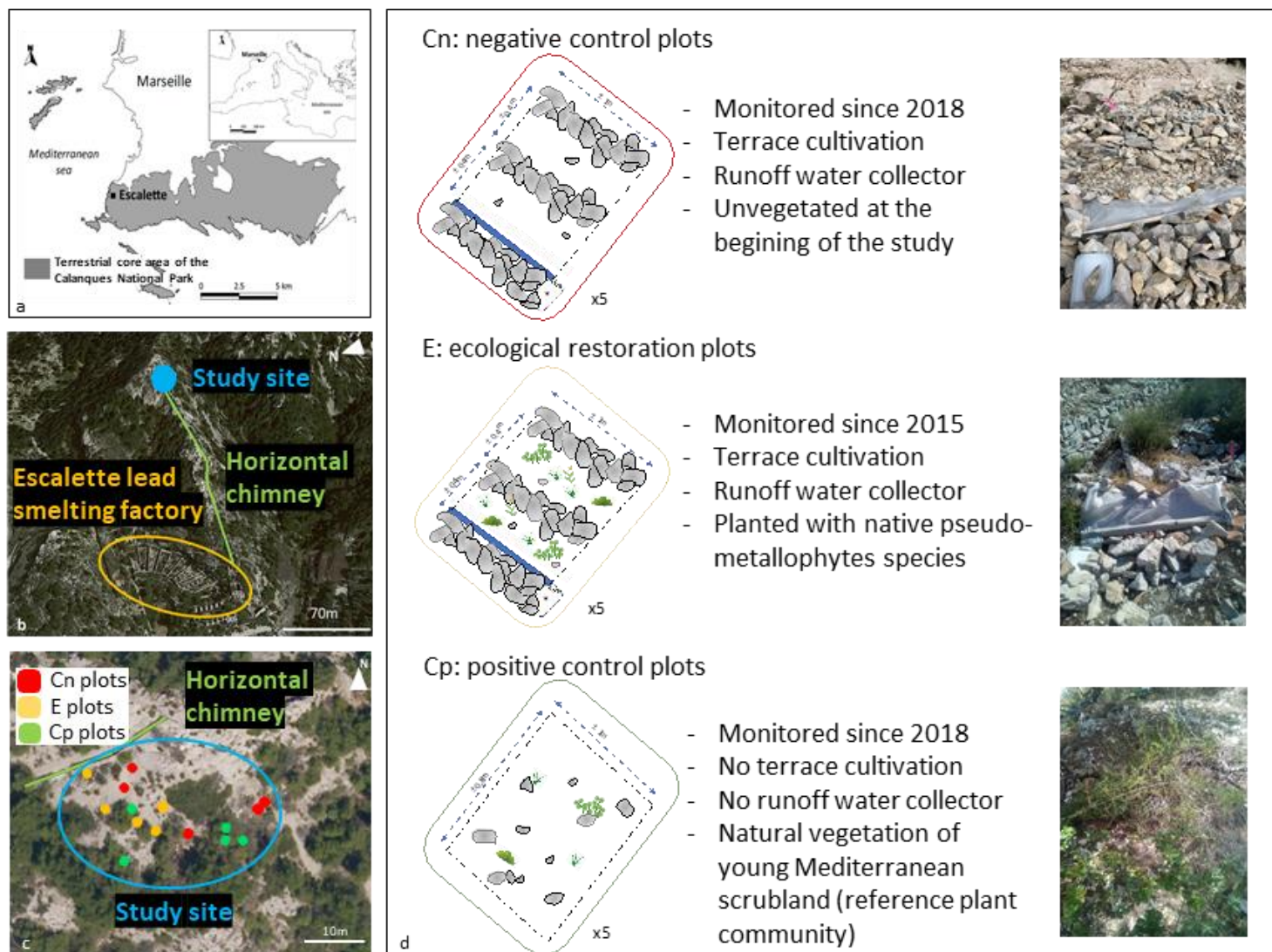
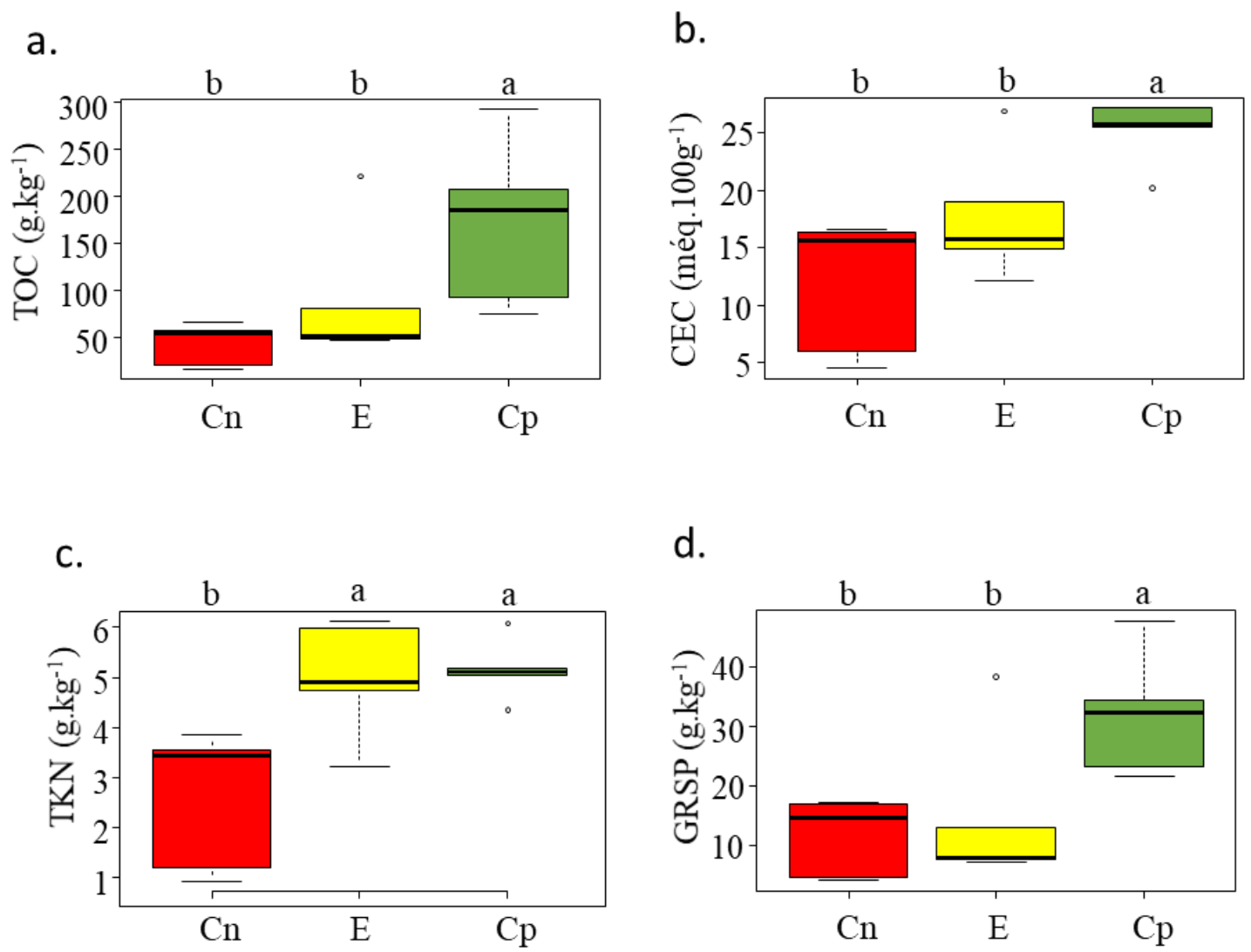


Figure2

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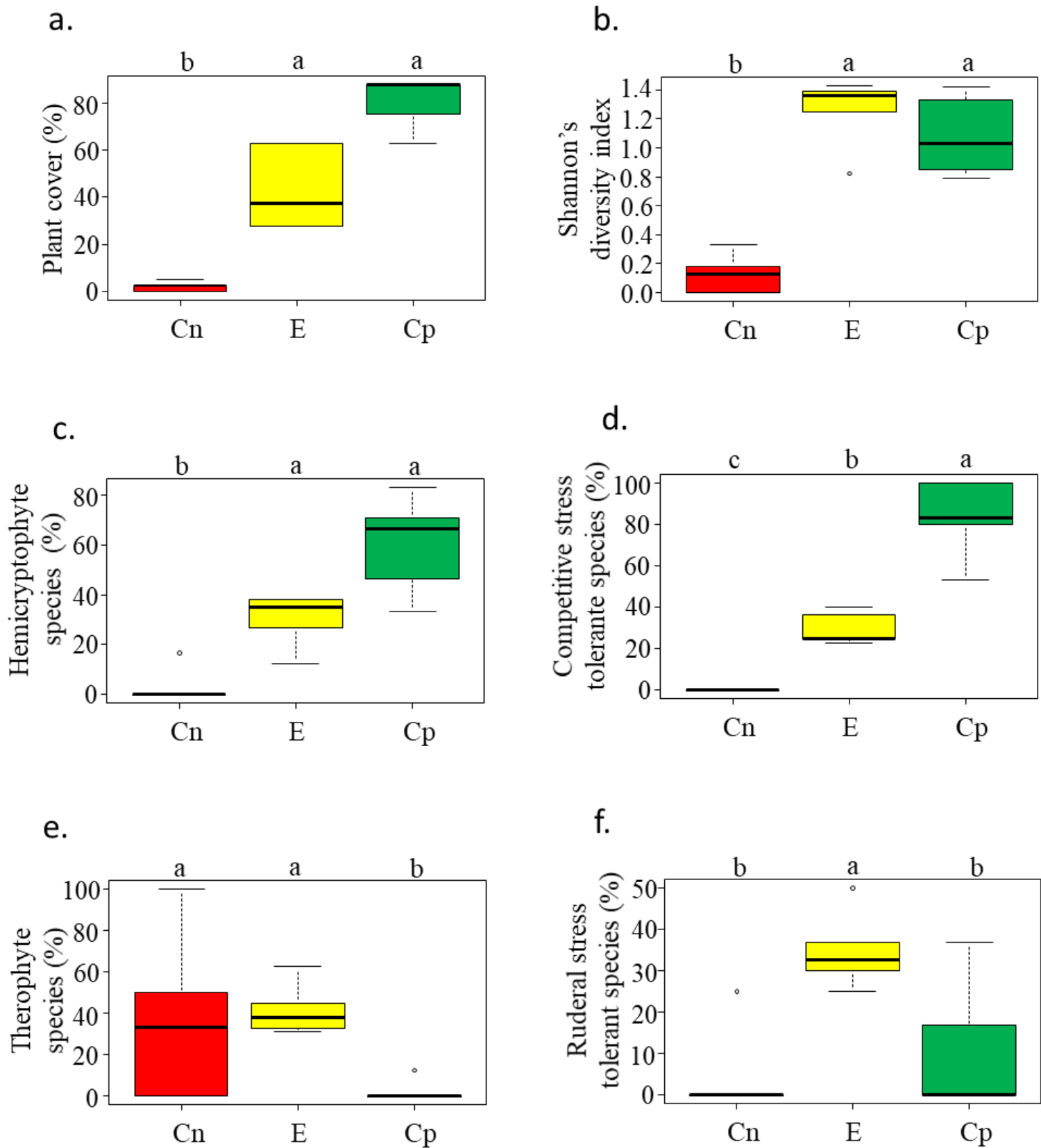


Figure4

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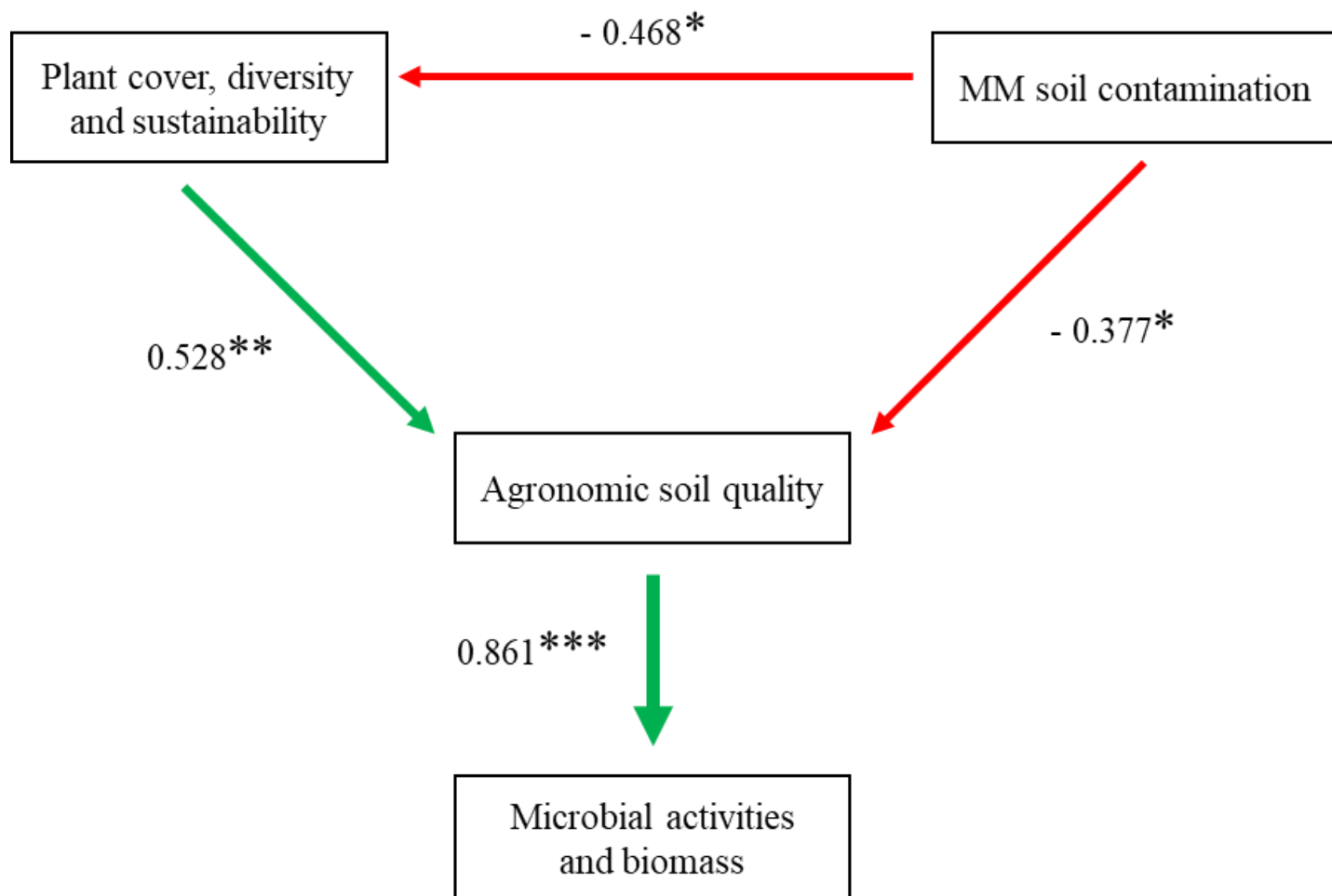


Figure5

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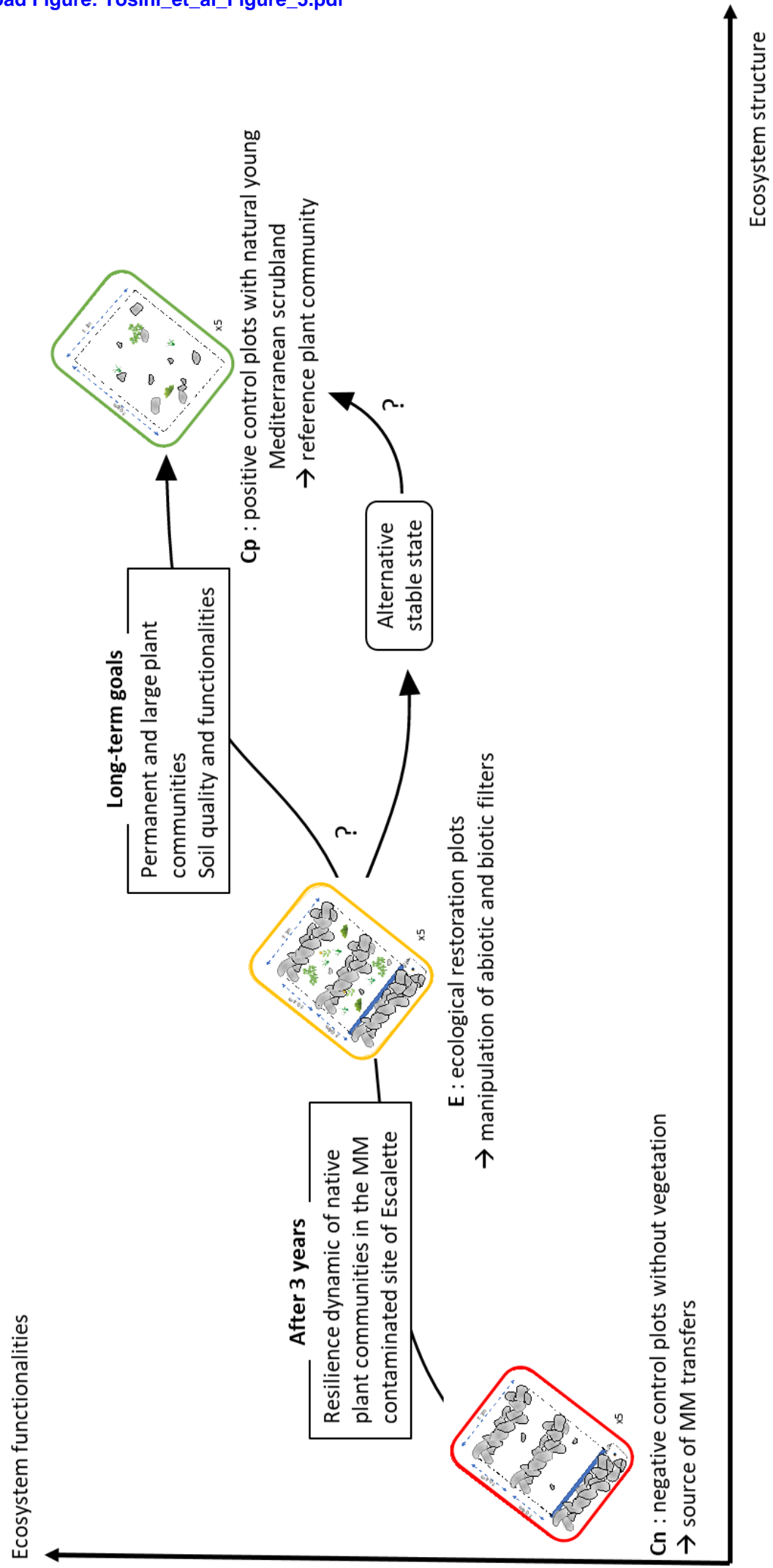


Table1

	Parameters	Cn plots	E plots	Cp plots
Soil characteristics	pH	7.68 ± 0.06 ^a	7.41 ± 0.11 ^{ab}	6.93 ± 0.15 ^b
	TOC (g.kg ⁻¹)	43.58 ± 10.25 ^b	90.28 ± 33.25 ^b	171.36 ± 39.58 ^a
	TKN (g.kg ⁻¹)	2.59 ± 0.63 ^b	4.99 ± 0.52 ^a	5.15 ± 0.27 ^a
	C/N	35.86 ± 4.53 ^a	24.58 ± 6.78 ^a	36.65 ± 6.35 ^a
	P (g.kg ⁻¹)	0.04 ± 0.00 ^a	0.04 ± 0.00 ^a	0.05 ± 0.01 ^a
	CEC (méq.100g ⁻¹)	11.84 ± 2.66 ^b	17.70 ± 2.53 ^b	25.12 ± 1.28 ^a
	Clay (%)	14.96 ± 2.75 ^a	21.24 ± 2.48 ^a	21.18 ± 3.75 ^a
	Lime (%)	19.65 ± 2.57 ^a	21.37 ± 1.83 ^a	17.65 ± 1.04 ^a
	Sand (%)	22.86 ± 3.29 ^a	18.02 ± 2.62 ^a	21.76 ± 1.65 ^a
	[As] _{tot} (mg.kg ⁻¹)	19770.04 ± 7082.15 ^a	13640.33 ± 1174.80 ^a	8579.00 ± 2250.43 ^a
	[Cu] _{tot} (mg.kg ⁻¹)	75.54 ± 15.98 ^a	60.51 ± 3.28 ^a	51.40 ± 5.73 ^a
	[Pb] _{tot} (mg.kg ⁻¹)	63470.68 ± 10902.03 ^a	75600.15 ± 3780.47 ^a	55685.76 ± 10653.43 ^a
	[Zn] _{tot} (mg.kg ⁻¹)	33754.54 ± 11515.45 ^a	25944.16 ± 2664.65 ^a	14457.50 ± 4061.24 ^a
	PLI	406.54 ± 107.11 ^a	351.45 ± 23.02 ^a	239.33 ± 50.23 ^a
	[As] _{mob} (mg.kg ⁻¹) (%)	3306.47 ± 987.35 (18.65 ± 1.49) ^b	3568.09 ± 423.65 (27.29 ± 5.12) ^a	2821.20 ± 312.74 (39.35 ± 7.27) ^a
	[Cu] _{mob} (mg.kg ⁻¹) (%)	26.62 ± 5.34 (35.28 ± 2.30) ^a	22.17 ± 0.78 (36.98 ± 1.73) ^a	21.04 ± 1.64 (41.62 ± 1.87) ^a
	[Pb] _{mob} (mg.kg ⁻¹)(%)	40357.01 ± 8421.02 (62.96 ± 6.78) ^a	45668.26 ± 2261.84 (60.67 ± 2.90) ^a	37579.61 ± 5272.05 (70.29 ± 3.97) ^a
	[Zn] _{mob} (mg.kg ⁻¹) (%)	11553.41 ± 3546.00 (36.17 ± 2.82) ^b	12398.93 ± 1075.45 (49.37 ± 5.10) ^a	8014.85 ± 1582.89 (63.34 ± 8.53) ^a
	SBR (µg CO ₂ -C.g ⁻¹ .h ⁻¹)	0.048 ± 0.024 ^b	0.042 ± 0.010 ^b	0.209 ± 0.024 ^a
	MB (µg C _{mic} .g ⁻¹)	0.033 ± 0.015 ^a	0.033 ± 0.011 ^a	0.095 ± 0.009 ^a
	qCO ₂ (µg CO ₂ -C.µg C _{mic} ⁻¹ .h ⁻¹)	2.237 ± 0.333 ^a	1.434 ± 0.147 ^a	2.224 ± 0.353 ^a
GRSP (g.kg ⁻¹)	11.42 ± 2.95 ^b	14.73 ± 5.98 ^b	31.75 ± 4.69 ^a	
Water runoff characteristics	As _{water} (µg)	16.09 ± 5.44 ^a	44.65 ± 20.80 ^a	NA
	Cu _{water} (µg)	3.06 ± 0.91 ^a	3.52 ± 2.47 ^a	NA
	Pb _{water} (µg)	47.79 ± 30.05 ^a	103.89 ± 65.44 ^a	NA
	Zn _{water} (µg)	489.72 ± 188.40 ^a	252.77 ± 40.16 ^a	NA
Plant communities characteristics	Plant cover (%)	2.00 ± 0.94 ^b	43.50 ± 7.87 ^a	80.00 ± 5.00 ^a
	Specific richness	0.60 ± 0.29 ^b	5.40 ± 0.51 ^a	4.00 ± 0.45 ^a
	Shannon's diversity index	0.13 ± 0.06 ^b	1.25 ± 0.11 ^a	1.08 ± 0.13 ^a
	Pielou equitability index	0.03 ± 0.03 ^b	0.75 ± 0.04 ^a	0.79 ± 0.03 ^a
	Geophyte (%)	0.00 ± 0.00 ^a	3.10 ± 3.10 ^a	7.00 ± 5.17 ^a
	Chamaephyte (%)	0.00 ± 0.00 ^b	25.02 ± 4.36 ^a	28.67 ± 5.13 ^a
	Hemicryptophyte (%)	3.33 ± 3.33 ^b	30.07 ± 4.87 ^a	60.17 ± 8.93 ^a
	Therophyte (%)	36.67 ± 18.56 ^a	41.81 ± 5.73 ^a	2.50 ± 2.50 ^b
	Phanerophyte (%)	0.00 ± 0.00 ^a	0.00 ± 0.00 ^a	1.67 ± 1.67 ^a
	Stress tolerant (%)	35.00 ± 18.71 ^a	28.00 ± 2.76 ^a	4.00 ± 2.45 ^a
	Competitive Stress tolerant (%)	0.00 ± 0.00 ^c	29.83 ± 3.53 ^b	83.33 ± 8.56 ^a
	Ruderal Stress tolerant (%)	5.00 ± 5.00 ^b	34.83 ± 4.24 ^a	10.67 ± 7.26 ^b
Competitive Ruderal Stress tolerant (%)	0.00 ± 0.00 ^a	5.33 ± 3.43 ^a	2.00 ± 2.00 ^a	